MOLECULAR BIOLOGY

* DNA - Genetic material - for atleast majority of organisms.
* Nucleic acid (polymer of) → nucleotides
*

Nucleic acids found in → Living system

2 types

[DNA]

- Acts as genetic material for most of the organisms.
- Most abundant genetic material

[RNA]

- Genetic material in some viruses.
- Mostly functions as messenger
- Adapter molecule
- Catalytic molecule

REPLICATION

DNA → (transcription) → mRNA → (translation) → protein

THE DNA

→ Long polymer of → deoxyribonucleotides

→ Length of DNA is → no. of nucleotides (or a pair of nucleotide (Base pairs)) present in it.

→ CHARACTERISTIC OF AN ORGANISM !!

1) ϕ x 174 (Bacteriophage) → 5386 nucleotides
2) Lambda (Bacteriophage) → 48502 bp
3) Escherichia Coli → 4.6×10^6 bp
4) Haploid content of Human DNA → 3.3×10^9 bp

* SS DNA → ϕ174, M13 → Bacteriophage
SS DNA → HIV → retrovirus
→ double copies of SS DNA

} DNA hi hota h

STRUCTURE OF POLYNUCLEOTIDE CHAIN

* Nucleotide — 3 components → A nitrogenous base, Pentose sugar, Phosphate group
Pentose sugar → DNA - Deoxyribose, RNA - Ribose

2 types of **Nitrogenous Bases**

Purines
1) Adenine
2) Guanine

Pyrimidines
1) Cytosine → common for DNA & RNA
2) Uracil → present in RNA only (at place of thymine)
3) Thymine → present in DNA

* A nitrogenous base is linked to OH of 1'C pentose sugar
→ ★N-glycosidic Linkage★ forms **NUCLEOSIDE**

DNA	deoxyadenosine	deoxyguanosine	deoxycytidine	deoxythymidine
RNA	Adenosine	Guanosine	Cytidine	Uridine

* When A phosphate linked to OH of 5'C of a nucleoside
→ ★Phosphoester linkage★ forms **Corresponding NUCLEOTIDE** → depending on type of sugar.
OR
Deoxynucleotide — in DNA

* 2 nucleotides are linked through 3'-5' phosphodiester linkage
↓ forms
DINUCLEOTIDE

* More nucleotides are joined in such manner to form polynucleotide chain.

* Polymer thus formed has
- at one end – Free phosphate moety at 5' end of sugar → 5' end of polynucleotide.
- at other end → Free OH group at 3'C of sugar → 3' end of polynucleotide chain.

* Backbone of a polynucleotide — formed due to → Sugar & phosphate

* Nitrogenous bases — linked to → sugar moiety
  → Project from the backbone

Thymine
↓
5-methyl Uracil

* In RNA, every nucleotide residue — has → additional -OH (at – 2'c position of Ribose)

FRIEDRICH MEISCHER — identified acidic nature of DNA in 1869.
↳ Named it - NUCLEIN

* Due to - technical limitation, in isolating such a long polymer intact the elucidation of structure of DNA remained elusive for a very long time.

* In 1953, James Watson & Francis Crick
  ↳ Based on - X ray diffraction data — prod. by → Maurice Wilkins, Rosalind Franklin
  ↳ Proposed — Double Helix - simple, but famous.
  ↳ One of the hallmark — Pairing b/w 2 strands of polynucleotide chains

* This proposition was also based on → ERWIN CHARGAFF'S
  For, ds DNA
  Ratio b/w Adenine and Thymine and Guanine and cytosine are constant & ≃ 1.

* Base pairing — confers → very unique property to polynucleotide chains.
  → complimentary to each other

* If each strand from DNA (parental) — acts as → template for synthesis of a new strand
  2 ds DNA produced (daughter) — would be → (identical to parent DNA)

* Genetic implication of structure of DNA became very clear.

★ Features of Double-Helix structure of DNA as follows —

(i) Made up of 2 polynucleotide chains

(ii) Backbone constituted by Sugar-phosphate

(iii) Bases ⟶ project inside

(iv) Two chains have anti-parallel polarity
- one chain – 5′ → 3′
- other chain – 3′ → 5′

(v) Bases are paired through H-bonds forming base pairs
(A ≡ C) (A = T)

(vi) Purines always come opposite to Pyrimidines
hence

(vii) This generates – approximately uniform distance b/w 2 strands of Helix.

(viii) Two chains coiled in ⟶ Right Handed Fashion

(ix) Pitch of helix ⟶ 3.4 nm

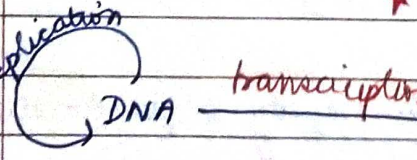
(x) Total bp ⟶ 10 bp in each turn

(xi) Distance b/w 2 bp ⟶ 0.34 nm

(xii) Plane of one bp stacked over ⟶ the other
+
(2) H-bonds
} ⟶ confers stability to helical structure

★ FRANCIS CRICK – Central Dogma Theory

★ Genetic information flows as DNA → RNA → protein



* In some VIRUSES – information can flow in reverse dir^n
RNA ⟶ DNA
(Reverse transcription)

PACKAGING OF DNA HELIX

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* Length of DNA double helix in a Typical mammalian cell → Total no of bp (diploid content) × distance b/w 2 Consecutive bp

$= 6.6 \times 10^9$ bp $\times$ 0.34×10^{-9} m/bp

$\approx$ 2.2 metres ~~imp~~

* Length of E. coli DNA, $\Rightarrow 4.6 \times 10^6 \times 0.34 \times 10^{-9}$

$\approx$ 1.36 mm DNA.

☆ In PROKARYOTES as → E. coli

- don't have defined nucleus
- DNA → not scattered throughout the cell.

DNA → -vely charged

held with some proteins that have +ve charges

in a region – NUCLEOID

→ DNA here is organised in large loops held by proteins.

☆ In EUKARYOTES → organisation is much more complex

→ has set of positively charged Basic protein → HISTONES

Proteins acquire charge depending on → Abundance of amino acid residues with charged side chains.

HISTONES rich in → Basic amino acid residues → lysine, arginine → carry +ve charge in their side chains.

organised to form → Histone Octamer – unit of 8 molecule.

-vely charge DNA is wrapped around → +vely charged HISTONE OCTAMER

→ NUCLEOSOME typically contains → 200 bp of DNA helix

constitute → Repeating unit of a structure of nucleus → CHROMATIN → Thread like, Stained coloured bodies

[Nucleosomes in chromatin] seen as → 'beads-on-string' structure

when viewed under ← ELECTRON MICROSCOPE

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30 million in mammalian cells

* Beads-on-strings structure in chromatin
  ↓ packaged to form
  CHROMATIN FIBRES
  ↓ which further gets → coiled / condensed } at metaphase stage
  CHROMOSOMES

* Packaging of chromatin at higher levels } requires → additional set of proteins
  → Non histone Chromosomal (NHC) proteins. ← collectively referred as

* In a typical nucleus, 2 regions are there

EUCHROMATIN	HETEROCHROMATIN
• Loosely packed	• More densely packed
• Stains light	• Stains dark
• Transcriptionally active chromatin	• Inactive

SEARCH FOR GENETIC MATERIAL

By 1926, quest to determine the genetic inheritance mechanism had reached molecular level.

Discoveries by Gregor Mendel, Walter Sutton, Thomas Hunt Morgan, Numerous other scientists } narrowed down that → Chromosomes are located in nucleus.

Transforming Principle

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* In 1928, Frederick Griffith in experiments with Streptococcus pneumoniae (bacterium responsible for pneumonia)
  → a living organism had changed (bacteria) in physical form

* Streptococcus pneumonia (pneumococcus) are grown on culture plate prod. → Smooth shiny colonies (S) (mucous (polysaccharide) coat)
  → Rough colonies (R)

* Mice infected with S strain (virulent) → die from pneumonia infection

* Mice infected with R strain → Do not die from pneumonia

Griffith was able to → kill bacteria by heating them.

S-strain (heat-killed) → inject into mice → Mice live

S-strain (heat-killed) + R strain (live) → inject into mice → Mice die

* He recovered living S bacteria from → dead mice

* R strain had somehow been → transformed by → heat killed S strain bacteria.

* Some "transforming principle" transferred from → heat killed S-strain → had enabled → R strain to synthesise
  - Smooth polysaccaride coat
  - Virulent

  → Must be due to transfer of genetic material.

However, by this experiment, Nature of genetic material was not defined

Biochemical Characterisation Of Transforming Principle

Prior to work of → Oswald Avery, Colin Macleod, Maclyn McCarty (1933 - 1944)

- Genetic material was thought to be a protein
- They worked to determine → Biochem. nature of "transforming principle" in Griffith's Experiment.
- They purified biochemicals → proteins, DNA, RNA] from → heat killed S cells
  - to see → which one could transform [live R cells into S cells]
    - they discovered → DNA alone from S bacteria caused → R bacteria to be transformed.
  - They discovered → RNA ases (RNA digesting enzyme), Protease (Protein " ") } did not affect → Transformation
    - So → RNA or protein wasn't the transforming substance
    - BUT → DNA ase did inhibit transformation.
    - hence, DNA - hereditary material

The Genetic Material is DNA

Unequivocal proof that → DNA is genetic material came from →
1. ALFRED HERSHEY
2. MARTHA CHASE

(1952)

worked with → Viruses that infect bacteria - BACTERIOPHAGES

BACTERIOPHAGES attaches to → Bacteria then → its genetic material enters → BACTERIAL CELL treats → Viral genetic material as if its own

Subsequently → manufactures more virus particles

They grew some viruses $\xrightarrow{\text{medium contained}}$ Radioactive phosphorus (^{32}P)

$\xrightarrow{\text{Others in medium}}$ Radioactive Sulphur (^{35}S)

(Bcz DNA has phosphorus but protein does not)

contained → Radioactive protein ★ / Non radioactive DNA

did not contain / contained → Radioactive protein / Radioactive DNA ★

(1)

* Radioactive phages $\xrightarrow{\text{were attached to}}$ E. coli $\xrightarrow{\text{As the infection proceeds}}$ Viral coats removed

(2) Virus particles were separated from Bacteria $\xleftarrow{\text{by}}$ agitating them in a blender $\xleftarrow{\text{by}}$ Bacteria $\xrightarrow{\text{from}}$

by → spinning them in a centrifuge

* Bacteria $\xrightarrow{\text{which was infected with}}$ Viruses (That had radioactive DNA) $\xrightarrow{\text{were}}$ Radioactive

DNA was material that passed from the virus to the Bacteria ← indicating

* One with Radioactive protein virus, infected Bacteria → not ~~radioactive~~

proteins did not enter bacteria from viruses. ← indicates

Properties Of Genetic Material

* In some viruses — RNA is genetic material *

Examples → Tobacco Mosaic Virus / QB bacteriophage

(4) Should be able to express itself in form of Mendelian characters

Molecule that can act as genetic material must fulfill

(1) should be able to generate its Replica (Replication)

(2) should be stable → chemically / structurally

(3) Should provide scope for slow changes (mutation) that are required for EVOLUTION

* Both nucleic acid (RNA, DNA)] due to → Rule of ① base pairing & ② complementarity

→ able to direct their duplications

* Proteins failed 1st criteria itself

* Genetic material should be → stable enough not to change with → different stages of ① life cycle ② Age ③ change in physiology of organism

* Stability as one of the → properties of genetic material was very evident in Griffith's experiment

↓

heat, which killed bacteria didn't kill ~~bacteria~~ some properties of Genetic material

this can be explained by → DNA, two strands being complement any if seperated by heating come together when appropriate conditions were provided.

* 2'-OH grp present at every nucleotide in RNA

↓ is

reactive grp

& makes → RNA labile & Easily degradable

catalytic ↓ hence reactive

* Therefore DNA chemically → Less reactive, Structurally more stable when compared to RNA

hence → DNA - BETTER GENETIC MATERIAL

* Presence of Thymine at place of Uracil also confers → ~~Additional stability~~ to DNA

understanding requires → process of REPAIR OF DNA → higher classes

* DNA, RNA } both → able to mutate

being → unstable → mutate at → faster rate

Consequently → Viruses with RNA genome → have → ① shorten life span & ② Evolve faster

* RNA → directly code for → synthesis of proteins → hence → can easily express the characters

* DNA → dependent on → RNA → for → synthesis of proteins

* Protein synthesising machinery → has evolved around → RNA

* Both DNA, RNA } can be used as genetic material but DNA being more stable → preferred for → storage of genetic information

* For Transmission of Genetic information → RNA is better

RNA WORLD

→ 1st genetic material

* Essential life processes → Metabolism, Translation, Splicing → evolved around → RNA

* RNA → acts as → Genetic material, catalyst → in some steps in living system

imp

* DNA → evolved from → RNA → With → Chemical modification that make it more stable

imp

being → double stranded & having complementary strand → further resists changes → by → Process of Repair

REPLICATION

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★ Watson & Crick — proposed

* Original Statement (1953) → It has not escaped our notice that specific pairing we have postulated immediately suggests a possible copying mechanism for genetic material.

→ Two strands — Seperate → act as template — for synthesis of → new complimentary strands.

→ After completion of Replication → each DNA molecule has → one parental, one newly synthesized strand.

→ This scheme — SEMICONSERVATIVE DNA REPLICATION

EXPERIMENTAL PROOF

→ First shown in — Escherichia Coli.
& then subsequently higher organism. as plants, animals

→ 1958 → Matthew Meselson & Franklin Stahl.

→ Grew — E. coli — in a medium containing $^{15}NH_4Cl$ (N^{15} – heavy isotope of nitrogen)
Only Nitrogen source for MANY GENERATIONS as

RESULT → N^{15} incorporated into → newly synthesized DNA, Nitrogen containing comp.

* This heavy DNA could be distinguished from normal DNA by

by Centrifugation in a (CsCl) density Gradient

N^{15} – Radioactive, – Heavy → hence can be → Seperated from N^{14} based on densities

→ Then they → transfered the cells into → Medium with normal $^{14}NH_4Cl$.

took samples at various definite time interval ← then

as cells would multiply

& → extracted DNA → that remained as → double stranded helices

★ Various samples were seperated independently on CsCl gradients to measure densities of DNA. ★

→ DNA extracted from culture after 1 generation (20 min) had → hybrid / intermediate density

After 2nd generation (40 min) was composed of → Equal amts of → hybrid DNA & light DNA.

* Very similar Experiments involving → Radioactive thymidine → newly synthesized DNA in chromosomes ← to detect

was performed on → Vicia Faba (Faba beans) → by → Taylor & colleagues → in 1958 (Also)

proved → DNA in chromosome replicate semiconservatively

THE MACHINERY & ENZYMES

* In living cells as → E. coli Replication requires → set of catalysts (enzymes) is

★ DNA dependant – DNA polymerase ★ since it uses a DNA template → to catalyse Polymerisation of Deoxynucleotides.

Highly Efficient enzymes as they have to catalyse → Polymerisation of large no. of nucleotides in a very short time

* E. coli —(completes replication in)→ 18 minutes
  - means → Average rate of Polymerisation → 2000 bp per sec.

* These polymerases —(have to be)→ ① fast ② high degree of accuracy to catalyze rxn

* Any mistake in replication —(results in)→ Mutation

* Replication → energetically expensive

* Deoxyribonucleoside Triphosphate —(serves dual purpose)→
  ① Acts as substrate
  ② provide energy for polymerisation rxn.
  - have 2 terminal phosphate are High energy phosphate —(same as in)→ ATP

* For Long DNA, 2 strands of DNA cannot be separated entire length
  - due to very high energy requirement

* Replication —(occur within)→ small opening of DNA helix → Replication fork.

* DNA dependent -DNA polymerase —(catalyse in)→ Only 1 direction 5' → 3'
  - this creates additional complications at replication fork

* One strand (template with polarity: 3'→5') —(replication is)→ Continuous

* Other template (template with polarity: 5'–3') —(replication is)→ Discontinuous

* Discontinuously synthesised fragments —(later joined by)→ DNA ligase

* DNA polymerases cannot → initiate process of Replication on their own

* Also, Replication don't → initiate randomly at any place in DNA.

there are definite regions in E.coli → where replication starts → ORIGIN OF REPLICATION

* Vectors provide → ORIGIN OF REPLICATION

* In Eukaryotes → replication of DNA at S phase

* Polyploidy → chromosomal anomaly

TRANSCRIPTION

→ process of copying genetic information → from one strand of DNA → into RNA

governed by → "Principle of Complimentarity" → EXCEPT THAT → A pairs with U

Replication	Transcription
One sets in, total DNA of organism get duplicated	Only "A segment" of DNA & only one strand copied into RNA

Q. Why both the strands are not copied during Transcription?

(1) If both strands acts as template → they would code for → RNA mol. with different sequences

in turn if they code for protein → sequence of AA → different

hence → 1 segment of DNA → would be coding → 2 different proteins

This would complicate ★ Genetic information transfer machinery ★

(2) 2 RNA formed → would be → complimentary to one another

hence would form → dsRNA → this would prevent → RNA from being translated

→ Transcription becomes futile.

TRANSCRIPTION UNIT

* 3 Regions in DNA :
  i) A promoter
  ii) The structural gene
  iii) A terminator

* DNA dependent - RNA polymerase —catalyze in→ 5'-3'

(3'T) → Template strand
↳ 3'-5'

5'-3' has → sequence same as RNA except (T at place of U)
↳ displaced during Transcription
Coding Strand
↳ but it codes for nothing

* All reference points while defining Transcription Unit is made with CODING STRAND

* If in Q. template strand given —then→ Complimentary (5'-3') RNA nikalna with U
* If in Q coding strand given —then→ same sequence likhna RNA me bs T ki jagah U kr dena. (5'-3')

* Promotor, Terminator } flank the → Structural gene in transcription unit.
* Promotor —located towards→ 5' end (Upstream) of Structural gene
* is a DNA sequence —that provides→ Binding site —for→ RNA polymerase
  Presence of it defines → Template & coding strands
* By switching positions with → terminator
* Direction of coding / template strand } → reversed.

* Terminator → located → 3' - end of Coding strand (Downstream)
  defined → end of transcription

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* There are → Additional regulatory sequence → that maybe present → Upstream or Downstream PROMOTER TO

TRANSCRIPTION UNIT & GENE

☆ Gene → Functional unit of inheritance
- located on → DNA
- also defined as → DNA sequence coding for → tRNA, rRNA

☆ Cistron → Segment of DNA coding for polypeptide.

Structural Gene
- Monocistronic
  - Eukaryotes
    - this monocistronic structural gene has interrupted coding sequences
    - Genes in eukaryotes → Split-gene
      - EXONS
        - Coding sequence
        - Expressed sequence
        - Appear in mature RNA or Processed
      - INTRONS
        - non-coding
        - intervening sequences
        - Do not appear in mature DNA
- Polycistronic
  - Bacteria
  - Prokaryotes
  - Mycoplasma

☆ Split gene arrangement → complicates definition of gene in terms of DNA segment.

☆ Inheritance of a character → affected by → Promotor sequence, Regulatory sequence of Structural gene.

hence ☆ Sometimes, Regulatory sequences loosely defined as ☆ Regulatory gene ☆ (even though these sequence CODE for nothing)

Types Of RNA & Process Of Transcription

* In Bacteria → 3 major types of RNA → mRNA, tRNA, rRNA → all needed to synthesise "proteins" in cell

- mRNA ↓ provides ★ template
- tRNA ↓ brings ★ amino acids & ★ Reads genetic code
- rRNA play structural role & catalytic role → during TRANSLATION

* Single → DNA dependent - RNA polymerase → catalyzes → transcription of all types of RNA in ★bacteria★

BACTERIAL TRANSCRIPTION

* RNA polymerase → binds to → Promotor → initiates → transcription
- uses → nucleoside triphosphates as → SUBSTRATE
- polymerises in ★ template dependent fashion ★ following "Rule of complimentarity."

* RNA polymerases —also facilitates→ opening of helix
  → counteracting elongation

* Only a short stretch of RNA —remain bound to→ enzyme

* Once polymerase —reaches→ Terminator region → (1) Nascent RNA falls off
  (2) RNA polymerase falls off
  → TERMINATION OF TRANSCRIPTION

* RNA polymerase —only capable of→ catalyzing process of elongation
  - associates with → σ factor (sigma) → initiation
  - associates with → ρ factor (rho) → Termination
  - association with these factor is transient. → alter specificity of the RNA polymerase.

* In bacteria, mRNA → does not require any processing to become active.
  - transcription, translation → occur in → Compartment- (no seperation of cytosol, nucleus) in bacteria
  - Coupled in Bacteria
  - bcz → translation can begin much before mRNA is fully transcribed.

EUKARYOTIC TRANSCRIPTION

(i) There are → 3 RNA polymerases. in → NUCLEUS
(in addition to RNA polymerase found in organelles)

* There is clear cut division of labour.

- RNA polymerase I → transcribes → r RNA's (28S, 18S, 5.8S)
- RNA polymerase II → transcribes → hn RNA (heterogeneous) → precursor of mRNA

RNA polymerase III
↓ transcribes
tRNA
5 srRNA
Sn RNA
(small nuclear RNAs)

RNA polym. I → rRNA
RNA polym. II → mRNA → tRNA
hnRNA
RNA polym. III

(ii) Complexity → Primary transcripts → contain ① exons & ② introns
↓ non-functional
hence undergoes SPLICING where ① introns are removed & ② exons joined in defined order

* hn RNA undergoes
  - Capping → unusual nucleotide (methyl guanosine triphosphate) $^{m}G_{PPP}$ → added to 5'-end of hnRNA
  - Tailing → Adenylate Residues (200-300 no.) → added to 3'-end of hnRNA → in TEMPLATE INDEPENDENT MANNER
  - Poly A tail

* ① Capping → ② Tailing → ③ Splicing
* Fully processed hnRNA → mRNA → is transported out of the Nucleus for translation
* Split Gene arrangement → ancient feature of genome
* Presence of Introns → reminiscent of antiquity
* Process of splicing → represents Dominance of RNA world.

GENETIC CODE

directs → sequence of AA → during → synthesis of proteins

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* No complimentarity exists b/w → nucleotides & amino acids
* Change in nucleic acid responsible for → Change in amino acids in proteins
* Deciphering of Genetic code req. → scientists from several field
  - ★ physicists
  - ★ Organic Chemists
  - ★ Biochemists
  - ★ Geneticists
* ★ GEORGE GAMOW, physicist argued → since there are 20 AA & only 4 bases
  - If they have to code → constitute combination of bases.
  - Suggested : Code should be made up of 3 nucleotides
    - would generate : 4^3 (4×4×4) = 64 codons. (more than req)
* No. of codons : (No. of bases)$^{\text{combination of base}}$
* PROVIDING PROOF, that Codon was TRIPLET :

(1) HAR GOBING KHURANA — instrumental in synthesizing RNA mol. with defined combination of bases
  - (1) homopolymers
  - (2) copolymers

(2) MARSHALL NIRENBERG — Cell free system for protein synthesis helped the code to be deciphered.

(3) SEVERO OCHOA ENZYME → Polynucleotide phosphorylase or
  - helpful in - polymerising RNA with defined sequences in TEMPLATE INDEPENDENT MANNER (enzymatic synthesis of RNA)

★ Features of Genetic Code :

(i) Codon - triplet.

(ii) 61 codons → code for → AA

3 codons → code for → nothing / no AA

(iii) Code is degenerate
(1 codon can code for some AA)

Stop codon / Terminator codons
UAA UAG UGA

(iv) Codon → is read in mRNA in → contagious fashion → no punctuations

(v) Non-ambiguous

(vi) non-overlapping

(vii) Code is → universal
(Eg: From Bacteria → humans, UUU - phenylalanine)

SOME EXCEPTIONS → found in → Mitochondrial codons
→ Some Protozoans

(viii) AUG - dual function
→ codes for → methionine (met)
→ acts as → initiator codon

Mutations & Genetic Code

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* Relationship b/w Genes, DNA } best understood by → Mutation

* Point Mutation → classical ~~function~~ Example : SICKLE CELL ANAEMIA

change in a single bp in gene for beta globin chain → Glutamate → Valine ← change of amino acid ← results in

* Insertion / Deletion of one or two bases → changes reading frame from point of insertion/deletion

* Such mutation → FRAMESHIFT INSERTION or DELETION MUTATION

* Insertion / deletion of "3" or "multiple of 3" in one or multiple codon hence one or multiple AA

unaltered from that point onwards ← reading frame remains

t RNA - Adapter Molecule

* AA don't have structural specialities to read the code uniquely.

* FRANCIS CRICK proposed → presence of adapter molecule

* t RNA before was called s-RNA (Soluble RNA)

* Ultimate expression of gene – protein form.

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specific for → Each AA

tRNA has → anticodon loop → has bases complimentary to the code

tRNA has → Amino acid acceptor end → AA binds to it

tRNA → For initiation there is → specific tRNA known as → initiator tRNA

* No tRNA for → stop codons
* Secondary structure of tRNA → clover leaf
* Actual structure compact molecule → inverted 'L'

TRANSLATION → process of polymerisation of AA to form a polypeptide

* Order / Sequence of AA are defined by → sequence of bases in mRNA

* AA joined by → peptide bond → Formation of this bond requires energy

(1) 1st step – Activation of AA | Charging of tRNA | Aminoacylation of tRNA

→ In presence of ATP → linked to their → cognate acids

* If 2 such charged tRNAs brough close enough → Formation of Peptide Bond favoured energetically

* Presence of catalyst enhance → Rate of peptide bond formation

* Ribosome → cellular factory for synthesising proteins

consists of → *Structural* RNAs + 80 different proteins

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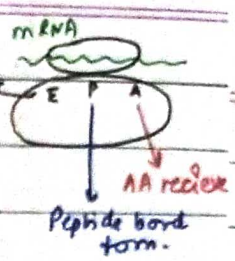
* IN INACTIVE STATE exists as → 2 subunits

large | small



* [Small subunit encounters mRNA] → PROCESS OF TRANSLATION begins

* Large subunit has "2" sites ① AA to bind ② Subsequent AA to be close enough to each other for formation of peptide bond.

* Ribosome also acts as → Catalyst for peptide bond formation (Ribozyme)

Eg. 23S rRNA (in bacteria) → Enzyme as well as Structural RNA

* A translational unit in mRNA is → sequence of RNA

flanked by → Start codon (AUG)

→ Stop codon

codes for a polypeptide

* mRNA has some additional sequences that are not translated → Untranslated Regions (UTRs)

present at both

5'-end (before start codon)

3'-end (after stop codon)

req. for efficient translation process

* INITIATION: Ribosome binds to mRNA at the start codon (AUG)

recognised only by initiator tRNA

* ELONGATION: (of protein synthesis) Complexes composed of AA linked to tRNA sequentially binds to appropriate codon in mRNA

by forming complementary base pairs with tRNA anticodon

Ribosome moves from codon to codon along mRNA

DNA → dictated by → Polypeptide sequence ← translated into ← AA are added one by one

Polypeptide sequence → represented by → RNA

END : Release factor binds to → stop codon → terminating translation → releasing → Complete polypeptide from → Ribosome

* RNA → first to evolve
* Hall mark of Double helix model — Hydrogen bonding b/w bases from opposite strands
* DNA replicates → semiconservatively guided by → complementary H-bonding
* [Segment of DNA that codes for → RNA] → GENE
* In bacteria → transcribed mRNA is functional hence can be directly translated.
* tRNA binds to → specific AA at one end & pairs through H bonding with → codes on mRNA through anticodon
* Translation evolved around → RNA indicating → life began around a RNA.
* Transcription, Translation → energetically expensive, tightly regulated
* In bacteria, (one gene) is → arranged together & regulated in units → called as → operons
* Lac operon — prototype operon in bacteria → regulation of enzyme synthesis by its substrate
* DNA fingerprinting (works on principle of polymorphism in DNA sequences) immense application → forensic science, genetic biodiversity, evolutionary biology

Regulation of Gene expression

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→ Results in : Formation of a polypeptide

→ Can be regulated at : several levels — In Eukaryotes — can be exerted at 4 levels

① transcriptional level. → formation of primary transcript

② processing level. → regulation of splicing

③ transport of mRNA (from nucleus) → to cytoplasm.

④ translational level.

- Genes in a cell — Expressed to → perform → A particular funct. OR Set of function.

Example — Glucose + Galactose

Enzyme – β-galactosidase (synthesized) ← E. coli — catalyse → Lactose (hydrolysis) → Glucose + Galactose → bact. use them → As a source of Energy

IF, no Lactose around bact. → then → they no longer require the synthesis of β galactosidase.

- Physiological cond. / Metabolic cond. / Environmental cond. → regulate → Expression of genes.

co-ordinated reg. of → Expression of several sets of genes → results in → Differentiation → Develop of embryo into → Adult organism

- IN PROKARYOTES →

Predominant site for control of Gene Expression — Control of the rate of transcriptional initiation

- In a Transcriptional unit → Activity of RNA polymerase at a given promoter.

Is in turn reg. by → interaction of accessory protein → effects its ability to → Recognise start sites.

- These Regulatory proteins act as

positively → Activators

negatively → Repressors.

Accessibility of promoter region of Prok. DNA → reg. by → Interaction of proteins → with sequences, termed as operators

Operator region adjacent to p... ele...

In most operons → And in most of the case → Sequence of operators → bind to → Repressor protein

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* Each operon has its specific → operator, repressor.

Example

In lac operon, there is specific → lac operator, lac repressor (interacts specifically with)

THE LAC OPERON

Elucidation of Lac operon → is a result of → Close associat... between A geneticist Francis Jacob, A biochemist Jacque Monod.

they first elucidated ... em

Transcriptionally regulated system.

refers to → lactose.

In LAC OPERON → A polycistronic structural gene → regulated by common → promotor gene, Regulatory gene.

Such arrangment → very common in bacteria → referred to as → Opuon.

Example: lac, trp, ara, his, val.

Lac operon → consists of
① One regulatory gene → i gene → inhibitor
② Three structural gene

z → codes for β-galactosidase (β-gal) → responsible for Hydrolysis of lactose

y → codes for permease → ↑ the permeability of cell to β-galacto sides.

a → codes for transacetylase

i → codes for repressor

* In most other operons as well the genes present in the operon are needed together to function in the same or related metab olic pathway.

Reg. for metabolism of lactose

Substrate for enzyme β-galactosidase → Lactose → reg. switching on/off of operon → hence termed → INDUCER

In the absence of preferred source glucose → If Lactose, provided in growth media of bact. → Lactose, transported into cells, through action of permease → Lactose then ind... the operon

Reg. of lac operon → violated as reg. of enzyme synthesis by its substrate. → Transcription proceeds

by repressor → -ve regulation

A very low level of expression of lac operon has to be present in the cell all the time, otherwise lactose cannot enter the cells.

The repressor of operon, synthesized, all the time (constitutive gene) → repressor binds to Operator region → Prevents, RNA polymerase from transcribing operon.

Reg. of lac operon is under +ve reg. as well but beyond syllabus.

In presence of Lactose / allolactose → Repressor is inactivated by interaction with inducer. → Allows, RNA polymerase access to promotor.

HUMAN GENOME PROJECT

Sequence of DNA → determines → Genetic information of a given organism / Genetic makeup.

- If 2 individuals differ → their → DNA sequences differ too (at least at some places) | These assumptions led to the → Finding out complete DNA sequence of human genome.

HGP → Launched in 1990 "Mega project"

Human genome → has → 3×10^9 bp.

- cost of sequencing 1 bp → US $ 3 → Total cost → 9 billion US $
- seq. obtained to be stored → 3300 books → each book has → 1000 pages → each page has → 1000 letters

- For a single human's genome [DNA sequences]

enormous amt of data expected to be generated, necessitated → use of high speed computational devices for → data storage, retrieval, analysis

closely associated with → BIOINFOMATICS (store data) (National centre for Biotechnology Information (NCBI)) (rapid develop. of a new area in biology) (science of analysing & organising collecting & analysing complex biological data such as genetic code)

GOALS OF HGP

- Identify approx. all 20,000 - 25,000 genes in human DNA.
- Determine sequence of 3 billion chemical bp that make up human DNA.
- Store this information in databases
- Transfer related technologies to other sectors, ex. Industries
- Improve tools for data analysis
- Address the ELSI (ethical, legal, social issues) that may arise from project.

HGP → 13 yr project → completed in → 2003 (actually in 2006)

additional contrib. of: ① Japan ② France ③ Germany ④ China

co-ord. by → US department of Energy, National Institute of Health

In the early yrs of project → Welcome trust (U.K) became major partner.

- Knowledge of effects of variation among individuals → leads to → Revolutionary new ways to → diagnose, prevent, treat → 1000's of disorders that affect human being.
- Learning about non-human organisms DNA sequences can lead to an understanding of → their → Natural capabilities → can be applied towards solving challenges → health care, agriculture, energy production, environmental remediation
- Non Human model that have been sequenced → ① bact., ② yeast, ③ caenorhabditis elegans (free living / non pathogenic nematode), ④ Drosophila (the fruit fly), ⑤ plants → Rice, Arabidopsis

Methodologies

2 Approaches

Focused on identifying all the genes that are expressed as RNA

↓

Expressed Sequence Tags (ESTs)

Blind approach of sequencing the whole set of genome that contained both → coding seq. → non-coding seq.

↓ later

Assigning diff. regions in the sequence with functions

Sequence Annotation — termed as

Steps

① Total DNA from a cell isolated → converting → ② DNA random fragments of relatively smaller sizes (bcz DNA → long polymer and there are technical limitation in sequencing very long pieces of DNA.

③ Cloned in suitable host → bact. / yeast using suitable vectors → BAC / YAC (Bact/Yeast Artificial chromosome)

④ Amplification of each piece of DNA fragments ← cloning resulted in

⑤ Fragments sequenced using Automated DNA Sequencers that worked on a principle of a method developed by Frederick Sanger. credited for → developing method for determination of AA sequence in proteins

⑥ These sequences were then arranged on some overlapping regions present in them

This req. generation of overlapping fragments for sequencing

Alignment of these sequences were humanly not possible

Specialised computer based programs were developed

Sequences, were subsequently annotated were → Assigned to each chromosome

May, 2006 ↓ Sequence of chromosome 1 completed (this was the last of the 24 human chr.) → 22 autosomes + X & Y chromosome

Another challenging task → assigning the genetic / Physical maps on genome

2 techniques to do it — using mf. on

Polymorphism of restriction endonuclease recognition sites (RFLP) Restricted fragment length polymorphism

Some repetitive DNA sequence (Microsatellites)

Salient Features of Human Genome

(i) Human genome → 3164.7 million bp.

(ii) Average gene consists of → 3000 bases

(iii) Size vary greatly, largest human gene → dystrophin → at 2.4 million bases.

(iv) Total no. of genes – 30,000 (much lower than previous estimates of 80,000 to 1,40,000 genes)
Almost all (99.9%) nucleotide bases are exactly the same in all people.

(v) The function are unknown for over 50% of the discovered genes.
* < 2% of genome codes for PROTEINS

(vi) Repeated sequence are stretches of DNA sequences that are repeated many times, sometimes hundred to thousands times.
They are thought to have no direct coding functions but they shed light on ① chromosome str. ② dynamics ③ evolution.

(vii) Chromosome 1 has most genes → 2968
Chr. Y has fewest genes → 231

(viii) Scientists have identified → 1.4 million locations where → single-base difference (SNPs) – single nucleotide polymorphism occur in humans ← this information.
Promises to revolutionise the processes of finding chromosomal locations for disease associated sequences & tracing human history.

Applications Of it & Future Challenges

Date ___/___/___

- Understanding of biological systems.
- Enabling a radically new approach to biological research.
- Approaching questions systematically and on a broader scale.
- They can study ~~all the genes in the genome~~. example → All the transcripts in a particular tissue/organ/tumour.
  → how tens of thousands of genes & proteins work together in an interconnected manner/networks to orchestrate chem. of life.

DNA fingerprinting

- 0.1% different base sequences makes → every individual unique in their phenotypic appearance.
- If one aims to find out genetic differences b/w → 2 individual / → among individuals of population sequencing the DNA would be daunting & expensive task. (3×10^6 bp – differences)
- Quick way to compare DNA sequences of any 2 individuals.

This process involves → Identifying differences in some specific regions in DNA sequence called Repetitive DNA.

In these → A small stretch of DNA repeated many times

open.

DNA
↓
density gradient centrifugation

Bulk DNA
- Major peak
- 99.9%
- Coding

Satellite DNA
- Small peaks
- 0.1%
- Non-coding (do not code for any protein)
- But form large portion of human genome.
- Show high degree of polymorphism & forms Basis of DNA fingerprinting.

Classified on basis of:
1) Length of segment
2) No of repeating units
3) Base comp. (A:T rich or G:C rich)

Mini satellite (10 – 60 bp) (VNTR)

Micro satellite (1 – 8 bp) (STR)

Polymorphism in DNA seq. Basis of → Genetic Mapping of human Genome. as well as DNA fingerprinting.

- Variation at Genetic level arise due to mutations → may arise in → Somatic cells OR Germ cells
  - Somatic cells → (through sexual reproduction) → TO other members of population
  - Germ cells → can transmit mutation → If germ cell mutation does not impair individuals ability to reproduce
- Allelic seq. Variation — DNA polymorphism occurs if more than one variant (allele) at a locus in human population with a frequency greater than 0.01.

DNA from every tissue → hair follicle
blood skin bone saliva sperm
from an individual

- shows same degree of polymorphism
  hence become very useful in identification tool in forensic applic.
- Polymorphisms are inheritable
  hence
- DNA fingerprinting is the basis of paternity testing in case of disputes.

- Inheritable mutation- observed in a popl^{n} at high freq
  ↓ referred to as
  DNA polymorphism

Date Page

probability of such variation to be observed in Non-coding DNA sequence is high bcz Mutations in these sequence may not have any immediate effect / impact in an individuals reproductive ability

These mutations keep on accumulating generation after generation & form one of the basis of variability / polymorph. ① ② For evolution & Speciation such polymorph. play an imp role.

diff types ranging from → Single nucleotide change to Very large scale changes.

ALEC JEFFREY
↓ developed
Technique of DNA fingerprinting
↓
Used satellite DNA as probe that shows very high degree of polymorphism.
called → VNTR (Variable Number of Tandeem Repeats)

this techn. involves → Southern blot hybridization using a radiolabelled VNTR as probe

① Isolation of DNA → ② Digestion of DNA by restriction endonuclease → ③ Separation of DNA fragments by electrophoresis.

④ transferring (Southern blotting) of sep. frag. of DNA to synthetic membranes → nitrocellulose / nylon

⑤ Hybridisation using labelled VNTR probe → radioactive ssDNA complementary to VNTR (imp)

⑥ Detection of hybridized DNA frag. by autoradiography

VNTR - A small DNA sequence
Arranged tandemly
For many copy numbers varies from → chromosome to chromosome in an individual.
Size varies from → 0.1 to 20 kb

* No of repeat shows → Very high degree of polymorphism

After hybridisation with VNTR probe → Autoradiogram gives → many bands of differing sizes. → These bands give → characteristic pattern for an individual DNA.

It differs from indiv. to indiv. except in case of monozygotic twins.

Sensitivity of this technique has been ↑ by use of PCR.

consequently → DNA from a single cell is enough to perform DNA fingerprinting analysis.

applications → On forensic science; determining population genetic diversities

* Currently, many different probes are used to generate DNA fingerprints.